Figure S1

A

Dox (ng/ml) 0 62.5 125 500

Ki67

Ki67/DAPI

B

control 2 hours 4 hours 12 hours

C

Cleaved caspase 3

Control Doxycycline

Cont 4h 8h 12h 16h Campt
Figure S2

A

0-20  1-19  2-18  4-16  6-14

B

PAX 7

Desmin

Myogenin

MYF 5

C

iC2C12-DUX4 (24 hours post induction)

fold difference

0 1.5

0 1.0 2.0 3.9 7.8 15.6 31.3 62.5 125 250 500 1000

doxycycline (ng/ml)
**Figure S3**

A

Dox 0 62.5 125 250 500 1000

B

![Graph showing fold difference vs doxycycline concentration]

C

![ACTIN DUX4 Western blot](https://example.com/)

D

Control AsAc + Dox β-ME + Dox MTG +Dox Vit K2 + Dox Dox
Figure S4

A

<table>
<thead>
<tr>
<th>Hours</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUX4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTIN</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

B

Control | Doxycycline

On MEFs

C

Cont | Dox

Actin Dux4

D

Control (EB day 10) | Dox for 3 days

Dox 0 25 50 75 125 250 1000

DUX4

ACTIN

Without MEFs

E

Control (EB day 10) | Dox for 3 days

Annexin V

AAD

F

Monolayer culture

No Dox | Dox for 2 days

EB differentiation

No Dox | Dox day 7-9

G

Dox from day:

0 14 no dox | Dox from day:

0 14 no dox

Ectoderm | Endoderm | Mesoderm
Figure S5

MyoD

Myf5

Myogenin
A. iC2C12-DUX4 & PAX3

<table>
<thead>
<tr>
<th>Pax3</th>
<th>Pax3/DAPI</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image of Pax3" /></td>
<td><img src="image2.png" alt="Image of Pax3/DAPI" /></td>
</tr>
</tbody>
</table>

B. iC2C12-DUX4 & PAX3

<table>
<thead>
<tr>
<th>hours (h)</th>
<th>doxycycline (ng/ml)</th>
<th>PAX3</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0.000004</td>
</tr>
<tr>
<td>12h</td>
<td>62</td>
<td>0.4</td>
<td>0.000008</td>
</tr>
<tr>
<td>12h-125</td>
<td>125</td>
<td>0.6</td>
<td>0.000012</td>
</tr>
<tr>
<td>12h-250</td>
<td>250</td>
<td>0.8</td>
<td>0.000016</td>
</tr>
</tbody>
</table>

C. iC2C12-DUX4 & PAX7

<table>
<thead>
<tr>
<th>Dox (ng/ml)</th>
<th>DUX4 &amp; GFP</th>
<th>DUX4 &amp; PAX3</th>
<th>DUX4 &amp; PAX7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.88</td>
<td>3.54</td>
<td>4.33</td>
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<td>62</td>
<td>7.85</td>
<td>13.2</td>
<td>6.4</td>
</tr>
<tr>
<td>125</td>
<td>11.7</td>
<td>17.1</td>
<td>13.1</td>
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<tr>
<td>250</td>
<td>8.7</td>
<td>22.8</td>
<td>17.9</td>
</tr>
<tr>
<td>500</td>
<td>8.7</td>
<td>22.8</td>
<td>17.9</td>
</tr>
<tr>
<td>1000</td>
<td>14.9</td>
<td>25.8</td>
<td>21.1</td>
</tr>
</tbody>
</table>
Figure S7

A

Doxycycline 0 ng/ml                      125 ng/ml                    500 ng/ml

B

ATP assay-24 hours

fold difference

0   125   250   500   1000   0   125   250   500   1000

iC2C12-DUX4               iC2C12-DUX4 & HOXB4